Plant Signaling & Behavior 4:9, 830-835; September 2009; © 2009 Landes Bioscience

Intracellular Ca^{2+} stores could participate to abscisic acid-induced depolarization and stomatal closure in *Arabidopsis thaliana*

Patrice Meimoun,† Guillaume Vidal,† Anne-Sophie Bohrer, Arnaud Lehner, Daniel Tran, Joël Briand, François Bouteau and Jean-Pierre Rona*

LEM (EA 3514); Université Paris Diderot-Paris7; Paris, France

†These authors contributed equally to this work.

Keywords: abscisic acid, anion channel, *Arabidopsis thaliana*, calcium, stomata

Abbreviations: Br-cADPR, 8-bromo-cyclic adenosine diphosphate ribose; $[Ca^{2+}]_{cyt}$, cytosolic calcium concentration; CICR, calcium-induced calcium release; IP_3, inositol-1-4-5-triphosphate; IP_3R, inositol trisphosphate receptor; PIP_2, phosphatidylinositol diphosphate membranaire; PI-PLC, phosphatidylinositol-phospholipase C; PM, plasma membrane; ROS, reactive oxygen species; RyR, cADPR/ryanodine receptor; V_m, plasma membrane potential

In *Arabidopsis thaliana* cell suspension, abscisic acid (ABA) induces changes in cytosolic calcium concentration ($[Ca^{2+}]_{cyt}$) which are the trigger for ABA-induced plasma membrane anion current activation, H^+-ATPase inhibition, and subsequent plasma membrane depolarization. In the present study, we took advantage of this model to analyze the implication of intracellular Ca^{2+} stores in ABA signal transduction through electrophysiological current measurements, cytosolic Ca^{2+} activity measurements with the apoaequorin Ca^{2+} reporter protein and external pH measurement. Intracellular Ca^{2+} stores involvement was determined by using specific inhibitors of CICR channels: the cADP-ribose/ryanodine receptor (Br-cADPR and dantrolene) and of the inositol trisphosphate receptor (U73122). In addition experiments were performed on epidermal strips of *A. thaliana* leaves to monitor stomatal closure in response to ABA in presence of the same pharmacology. Our data provide evidence that ryanodine receptor and inositol trisphosphate receptor could be involved in ABA-induced (1) Ca^{2+} release in the cytosol, (2) anion channel activation and H^+-ATPase inhibition leading to plasma membrane depolarization and (3) stomatal closure. Intracellular Ca^{2+} release could thus contribute to the control of early events in the ABA signal transduction pathway in *A. thaliana*.

Introduction

The plant hormone abscisic acid (ABA) is involved in regulation of plant development and adaptation to various environmental stresses.[1] Under unfavourable conditions, such as cold, salinity or water shortage, ABA activates a complex signaling pathway leading to the expression of ABA-responsive genes and to the stomatal closure which limits water loss through transpiration and prevents dehydration.[2-4] Stomatal closure represents one of the fastest ABA responses arising in a few minutes. This closure is accomplished by the release of anions and K^+ and by metabolic degradation of the major organic anion malate.[3,4] ABA induces plasma membrane (PM) depolarization of the guard cells initiated by anion current activation.[3-7] The fast depolarization of PM leads to the activation of outward rectifying K^+ currents and the inhibition of inward K^+ channels.[3] These changes in guard cell ion transport are responsible for cell shrinkage and stomatal closure. Further events such as lipid-based signals,[8] reactive oxygen species,[3] nitric oxide (NO)[9] and G proteins[10] were shown to modulate ion fluxes and proposed to play a role in ABA-induced stomatal closure. Abscisic acid also provokes a fast rise in $[Ca^{2+}]_{cyt}$,[11] which may act as second messengers leading to guard cell closure.[12] Although the role of Ca^{2+} in stomatal closure is still a matter of question,[13] there are remarkable parallels in the effects of ABA and Ca^{2+} on guard-cell transport processes. Like ABA, experimental elevation of Ca^{2+} levels inhibits the H^+-ATPase,[14] inhibits the inward K^+ channels and activates anion channels[3] initiating the PM depolarization. Influx of Ca^{2+} through PM calcium channels was reported on guard cells.[3,15] However, ABA-induced $[Ca^{2+}]_{cyt}$ increases may also arise by release from intracellular stores. Two Ca^{2+} channel/receptor are supposed to control the release of Ca^{2+} to the cytosol during signal transduction, inositol trisphosphate receptor (IP_3R) and ryanodine/cADPR receptor (RyR),[16,17] although no homologous genes have yet been found for these receptors in plants. In animal cells, IP_3R and RyR are

*Correspondence to: Jean-Pierre Rona; Email: jean-pierre.rona@univ-paris-diderot.fr
Submitted: 06/09/09; Accepted: 06/30/09
Previously published online: http://www.landesbioscience.com/journals/psb/article/9396

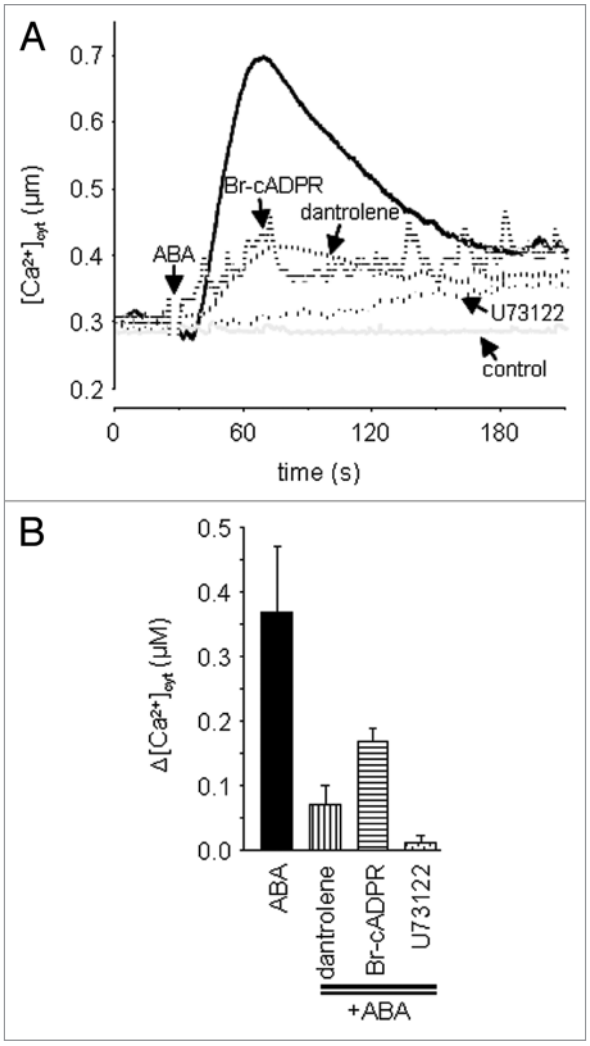

Figure 1. (A) Changes in $[Ca^{2+}]_{cyt}$ of Arabidopsis cells expressing the apoaequorin gene upon 10 µM ABA addition, alone or after a 30 min pretreatment with intracellular store Ca^{2+} channel inhibitors 100 µM dantrolene, 100 µM Br-cADPR, or 10 µM U73122. Controls with H_2O were performed. (B) Mean values of $\Delta[Ca^{2+}]_{cyt}$ upon 10 µM ABA addition, alone of after pretreatment with intracellular store Ca^{2+} channel inhibitors. Results correspond to the means of at least four independent experiments ± standard errors.

known as CICR channels.[18] In guard cells, the involvement of PI-PLC has been demonstrated in ABA-induced Ca^{2+} increase and stomatal closure.[19] PI-PLC hydrolyses PIP_2 to IP_3, the second messenger which activates the IP_3R leading to Ca^{2+} release from intracellular stores.[12] The activation of ADPR cyclase was suggested to be an early ABA-signaling event and that an increase in cADPR plays an important role in downstream molecular and physiological ABA responses.[17,20] The injection of cADPR was shown to induce Ca^{2+} rises, suggesting a role for cADPR as intermediate of the ABA-induced stomatal response in *C. communis*.[21] Our aim was to analyze if Ca^{2+} release from intracellular stores were involved in ABA-induced depolarization due to the activation of anion channels and inhibition of the H^+-ATPases in *A. thaliana*.

One way to address this issue would be to measure, in the same cells, both $[Ca^{2+}]_{cyt}$ and the relevant ionic current responses. In *A. thaliana* cell suspension, ABA induces changes in $[Ca^{2+}]_{cyt}$ which are the trigger for ABA-induced anion currents activation, plasma membrane H^+-ATPase inhibition, and consequently PM depolarization.[22] ABA-induced H_2O_2 production contributes to the PM depolarization through a $[Ca^{2+}]_{cyt}$ increase[23] as in guard cells.[24] Although, the involvement of intracellular Ca^{2+} stores remains unknown, this model appears thus peculiarly convenient to combined Ca^{2+} measurements, with transgenic cells expressing the apoaequorin gene, electrophysiological measurements and external pH measurement representative of H^+-ATPase activity.[22] Experiments were conducted in presence of specific inhibitors of the Ca^{2+}-receptor/channel RyR (Br-cADPR, dantrolene[25]) and IP_3R (U73122,[26]). We further compared suspension cells and guard cells signaling pathways by using the same pharmacology on ABA-induced stomatal closure on epidermal strips of *A. thaliana* leaves. Our results clearly provide strong evidence that in *A. thaliana* different intracellular Ca^{2+} stores could participate to the ABA-induced depolarization due to anion channel activation and H^+-ATPase inhibition in suspension cells which may also be involved in stomatal closure.

Results and Discussion

ABA-induced changes in $[Ca^{2+}]_{cyt}$ are dependent on Ca^{2+} release from intracellular stores. The ABA-induced $[Ca^{2+}]_{cyt}$ increase previously observed[22,23] was confirmed in freshly generated *A. thaliana* suspension cells expressing apoaequorin addressed in the cytosol. Specific inhibitors of two major channel/receptor complexes from intracellular stores, the RyR, an intracellular Ca^{2+} channel triggered by cADPR, and the IP_3R triggered by IP_3, were used to show the implication of these Ca^{2+} stores in $[Ca^{2+}]_{cyt}$ changes; Br-cADPR and dantrolene, RyR antagonist[27] which modulates the channel function inhibiting the Ca^{2+} release and U73122, inhibitor of PI-PLC activity (hydrolysis of PIP_2 to IP_3) which activates the IP_3R. In 60% of the experiments ABA induced an increase in $[Ca^{2+}]_{cyt}$ of about 0.37 ± 0.1 µM during 2.5 min after the addition of 10 µM ABA in the medium (**Fig. 1**). In this freshly generated aequorin suspension cells, the ABA induced changes in aequorin luminescence present only one spike peaking at about 40 s and was not a biphasic event as previously reported.[22,23] Immediate spikes, such as the one reported in Brault et al.[22] are sometime considered as non-specific in aequorin technology.[28] However, this discrepancy could also reflect a difference in sensitivity of these cell suspensions to ABA. In *C. communis* guard cells at high ABA concentration, Ca^{2+} influx could make a major contribution to an increase in $[Ca^{2+}]_{cyt}$ but at low ABA the intracellular release, which could present a different kinetic, makes the major contribution.[15] The redox state could also switch the conditions where extracellular Ca^{2+} is used as a primary supply to conditions where intracellular Ca^{2+} stores are mobilized. Thus in ABA-induced responses the origin of the increase in $[Ca^{2+}]_{cyt}$ could have relative importance depending on conditions. We could also argue that cell suspensions could use distinct and different receptor types and/or signal transduction pathways in ABA as observed for

the differential ABA-induced regulation of *V. faba* K^+ channels between mesophyll and guard cells.[29] However, the increase in $[Ca^{2+}]_{cyt}$ we observed (**Fig. 1**) was considerably inhibited when cells were pre-treated with 100 µM Br-cADPR (decrease of 54%) and dantrolene (decrease of 80%) or totally suppressed with 10 µM U73122 (**Fig. 1B**). Our results suggest that ABA-induced Ca^{2+} releases through IP_3R like and RyR like occur in our model in accordance with data observed on guard cells.[12,21] Even if the activation of ADPR cyclase was suggested to be an early ABA-signaling event and that an increase in cADPR plays an important role in downstream molecular and physiological ABA responses,[17,20] it cannot be excluded that the observed effects of inhibitors may be indirect since no homologous genes have yet been found for ryanodine and IP_3 receptors in *A. thaliana*.

ABA-induced depolarization and anion channel activation are dependent on the IP_3R and the RyR. In accordance with our aim, an electrophysiological approach was undertaken in order to determine the putative role of Ca^{2+} release from intracellular stores on ABA-induced PM depolarization and increase in anion currents, previously characterized in our model[30] and in response to ABA.[22] As previously reported[22,23] 10 µM ABA induced a depolarization of about +15 mV (**Fig. 2F**) in about 50% of the cells. The effect of ABA was tested in presence of Br-cADPR or dantrolene, two antagonists of the RyR, or U73122. Pre-treatment of Arabidopsis cells with this pharmacology abolished the ABA-induced depolarization (**Fig. 2F**) in 80% of the cells for Br-cADPR and dantrolene and 100% of the cells for U73122. Anion current increase triggered by ABA (166.5 ± 12.9%) (**Fig. 2A and E**) was also abolished or drastically reduced in presence of dantrolene (15.3 ± 15.5%) (**Fig. 2D and E**), Br-cADPR (55.3 ± 12.5%) (**Fig. 2C and E**) or U73122 (75.2 ± 9.1%) (**Fig. 2D and E**). These results strongly suggest that in *A. thaliana* suspension cells the Ca^{2+} release through RyR and IP_3R could occur upstream of the increase in anion currents and subsequent PM depolarization (**Fig. 2F**) in the ABA signaling pathway, in accordance with data observed on guard cells from *C. communis*[15] and oppositely to what observed in *V. faba* guard cells.[7]

ABA-induced extracellular alkalization involves the IP_3R and the RYR. In *A. thaliana* cell suspension, ABA-induced depolarization is also partly due to PM H^+-ATPase inhibition which induced a rapid medium alkalization.[22] The pH of the culture medium in cultured Arabidopsis cells was thus monitored in presence of dantrolene or U73122 (**Fig. 3**). **Figure 3A** shows the alkalization of medium pH upon 10 µM ABA addition (ranged from 0.18 ± 0.02 pH units in less than 45 min). When cells were pretreated with dantrolene or U73122, the ABA-induced alkalization was decreased of 15% and 36%, respectively (**Fig. 3B**). As previously discussed[22] inhibition of H^+-ATPases by an increase in $[Ca^{2+}]_{cyt}$ were previously reported for different plant materials. Our data suggest that ABA-induced Ca^{2+} release from intracellular stores could participate in the decrease of PM H^+-ATPase activity, as observed for *V. faba* guard cells,[31] leading thus to the depolarization of the cells and the alkalization of the medium. However, the relatively weak percentages of inhibition

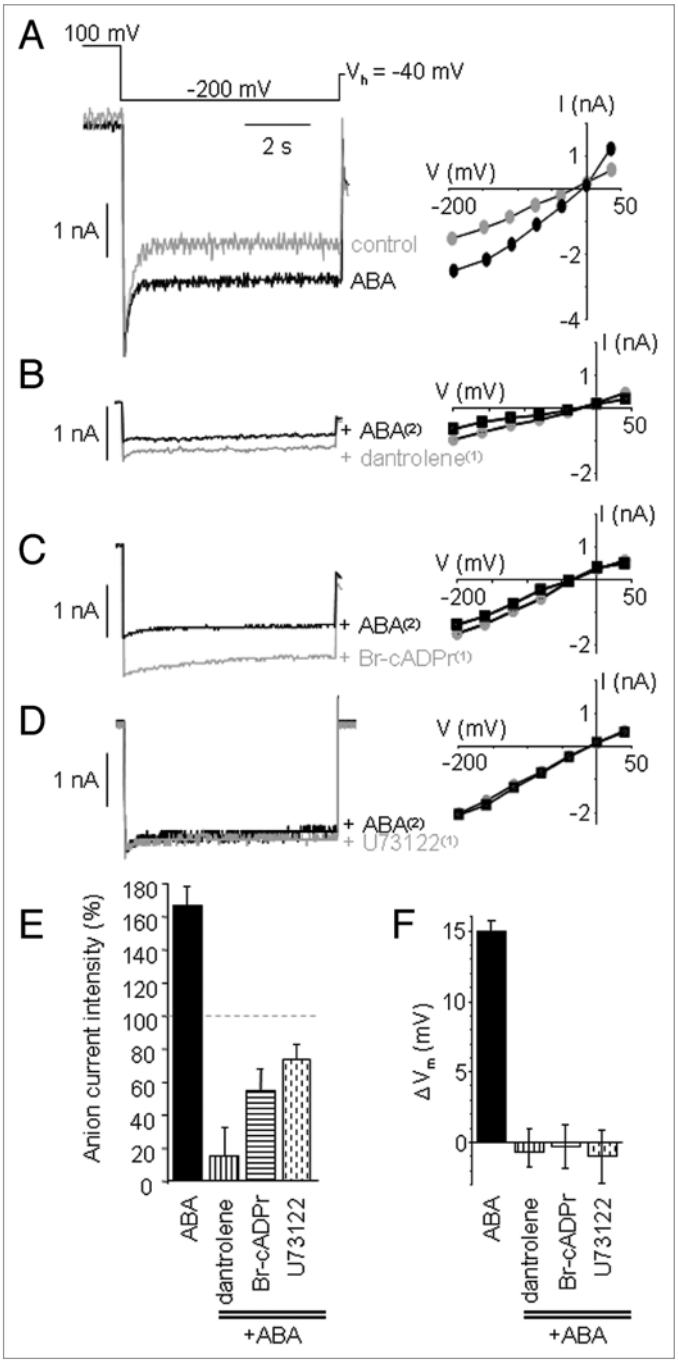

Figure 2. (A) Typical activation of anion currents by ABA. Anion currents measured under control conditions and after adding 10 µM ABA. Holding potential (V_h) was -40 mV. The current amplitudes (at 6.3 s) were measured for membrane potentials ranging from -200 to +40 mV before and after ABA addition. (B–D) Anion currents recorded under -200 mV pulse before(1) and after(2) the addition of 10 µM ABA in presence of the inhibitors (100 µM dantrolene, 100 µM Br-cADPR, or 10 µM U73122). (E) Mean steady state values of anion current intensity (% of control) recorded at -200 mV at maximal depolarization upon 10 µM ABA addition, alone or after pretreatment with intracellular store Ca^{2+} channel inhibitors. Dotted line corresponds to control level for each effector without ABA. Results correspond to the means of at least three independent experiments ± standard errors. (F) Mean values of plasma membrane potential (ΔV_m) recorded at maximal depolarization upon 10 µM ABA addition, alone for the 50% responding cells or after treatment with inhibitors. Results correspond to the means of five independent experiments ± standard error.

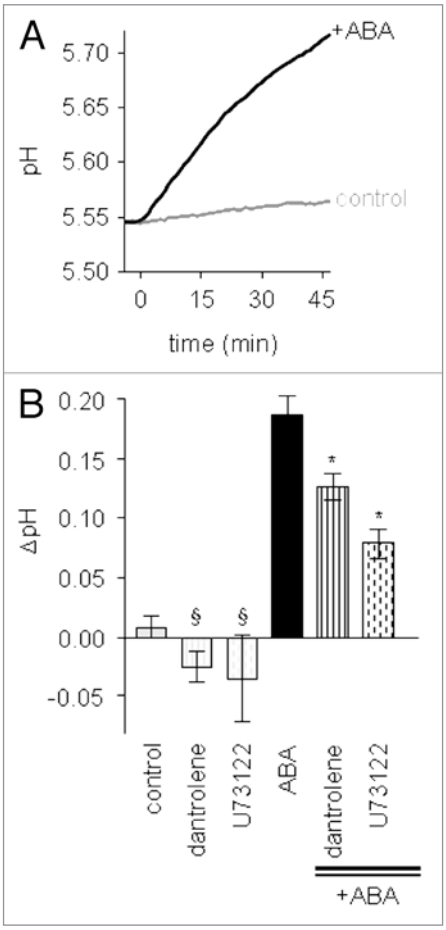

Figure 3. (A) Typical medium alkalization induced by ABA (10 μM) in *A. thaliana* suspension cells. Representative result of six independent experiments is shown. (B) Mean values of ΔpH correspond to pH variation after 45 min in presence of intracellular store Ca^{2+} channel inhibitors (100 μM dantrolene or 10 μM U73122) with or in absence of ABA (10 μM). The initial pH ranging from pH 5.4 to 5.6 could be modified by 0.4 upH depending on pharmacology. Results correspond to the means of six independent experiments ± standard errors. §Not significantly different and *significantly different, $p < 0.05$.

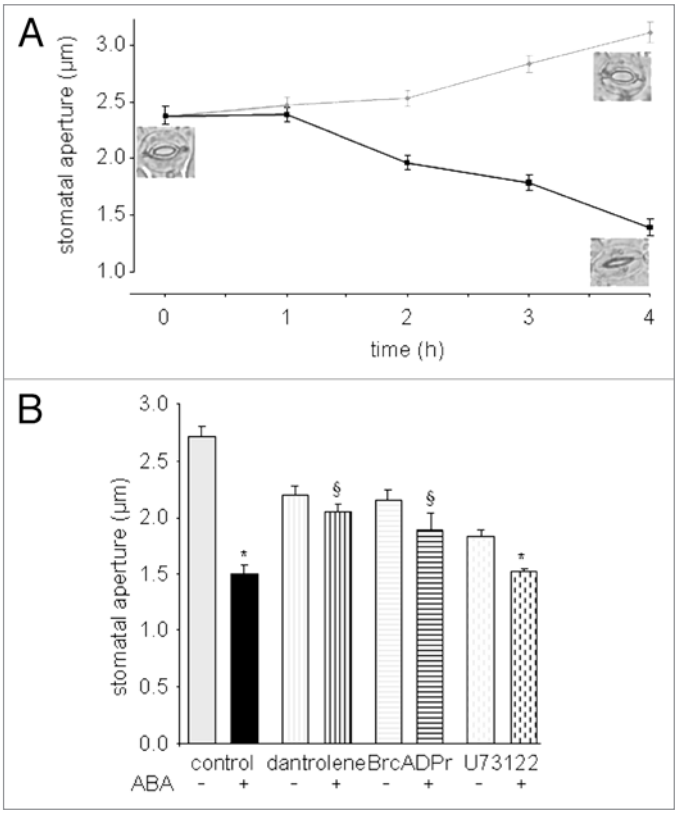

Figure 4. (A) Time-course changes in Arabidopsis stomatal apertures (width, μM) in presence (black) or absence (grey) of ABA (10 μM). (B) Mean values of stomatal apertures with intracellular store Ca^{2+} channel inhibitors in presence (+) or absence (-) of ABA (10 μM). Inhibitors were added 30 min before ABA addition (100 μM dantrolene, 100 μM Br-cADPR, or 10 μM U73122), apertures were recorded four hours after ABA treatment. Results correspond to the means of four independent experiments ± standard errors. §Not significantly different and *significantly different, $p < 0.05$.

could indicate that only a part of the cell population responds through a Ca^{2+} dependant way. If H_2O_2 production, after ABA stimulation of the NADPH-oxidase in suspension cells,[23] could also participate to the medium alkalization,[32] we can not rule out that some cells undergo a Ca^{2+}-independent alkalization of the medium.

ABA-induced stomatal closure is dependant of intracellular Ca^{2+} stores. The three sets of data we recorded in response to ABA in suspension cells, namely increase in $[Ca^{2+}]_{cyt}$, anion channel activation and H^+-ATPase inhibition, likely occur in guard cells.[3,13] In order to integrate our data in physiological stomata regulation in *A. thaliana*, we checked the involvement of intracellular Ca^{2+} stores in the ABA signaling pathway. Thus, we monitored the stomatal closure using the same specific pharmacology. Epidermal strips were prepared from the abaxial surface of intact leaves (similar size and developmental stage) in which we could measure stomatal aperture. Time course observation showed that after four hours under light and in presence of ABA, the pore width of stomata decreased to 1.40 μm (**Fig. 4A**) corresponding to 60% less than the control. Using the same experimental condition, we examined the effect of dantrolene, Br-cADPR and U73122 on the ABA-induced stomatal closure (**Fig. 4B**). In presence of these inhibitors, the mean stomatal apertures after ABA treatment suggest that stomatal closure was strongly inhibited (**Fig. 4B**). It is to be noted that, as previously observed for the three sets of data, a percentage between 5 and 10% of the *A. thaliana* guard cells responded to ABA by a stomatal closure even in presence of RyR or IP_3R inhibitors. However, the effect of U73122 indicates that IP_3R could be involved in the ABA physiological process leading to stomatal closure as previously reported in *C. communis*.[12,19] In the same way, our data suggest a role for cADPR as intermediate of the ABA-induced stomatal response in *A. thaliana* as shown on *C. communis*.[21]

Material and Methods

Plant material. *Arabidopsis thaliana* L. was grown from seed in an environmentally controlled chamber (eight hour photoperiod,

under 100 µmol photons m^{-2} s^{-1} at the leaf level, 24 ± 2°C) and plants were weekly watered.

Preparation of epidermal strips. *A. thaliana* leaves from 4–6 week old plants were harvested on hour after the beginning of the light period. Epidermal strips were carefully prepared from abaxial epidermis then placed cuticule side-down on microscope slides covered with medical adhesive (Dow Corning 355, Peters surgical)[33] and immediately floated in 10 mM MES pH 6.1, 50 mM KCl, 1 mM $CaCl_2$ (opening buffer) under white light (40 µmol photons m^{-2} s^{-1}) for three hours before future treatments.

Stomatal opening measurements. Epidermal strips were analyzed with a Laborlux S (Leica, Germany) microscope (x400). For quantifying, microscope fields were digitalized with a Kappa CF11DSP (Nikon, Japan) digital camera. The width of the stomatal aperture was measured using the image analysis software Metreo Kappa Image Base (Kappa, Germany). The pore width from at least 200 stomata per treatment per experiment was measured and pooled together for statistical analysis. Data are expressed as µm and are means ± SE.

Cell culture conditions. *A. thaliana* L. suspension cells were grown in Gamborg medium (pH 5.8). They were maintained at 24 ± 2°C, under continuous white light (40 µmol photons m^{-2} s^{-1}) and continuous shaking (gyratory shaker) at 120 rpm. Suspensions were sub-cultured weekly using 1:10 dilution. All experiments were performed at 24 ± 2°C using log-phase cells (4 days after sub-culture).

Aequorin luminescence measurements. Cytoplasmic Ca^{2+} variations were recorded with freshly generated *A. thaliana* cell suspension expressing the apoaequorin gene.[22] For calcium measurement, aequorin was reconstituted by overnight incubation of cell suspension in Gamborg medium with 2.5 µM native coelenterazine. Cell culture aliquots (200 or 500 µL) were transferred carefully into a luminometer glass tube, and the luminescence counts were recorded continuously at 0.2 s intervals with a FB12-Berthold luminometer. Treatments were performed by pipette injection of 10 µL of ABA. At the end of each experiment, the residual aequorin was discharged by addition of 500 µL of a 1 M $CaCl_2$ solution dissolved in 100% methanol. The resulting luminescence was used to estimate the total amount of aequorin in each experiment. Calibration of calcium measurement was performed by using the equation: pCa = 0.332588(-logk) + 5.5593, where k is a rate constant equal to luminescence counts per second divided by total remaining counts.[22] Data are expressed as µM and are means ± SE.

Electrophysiology. Cells were impaled in the culture medium with borosilicate capillary glass (Clark GC 150F) micropipettes (resistance: 50 MΩ when filled with 600 mM KCl). Main ion concentrations in the medium after 4d were 9 mM K^+, 11 mM NO_3^- [35]. Individual cells were voltage-clamped using an Axoclamp 2B amplifier (Axon Instruments, Foster City, CA,) as previously described.[22,23,30] Data are expressed as mV or % and are means ± SE.

Extracellular pH measurements. Extracellular pH was measured directly in the medium.[22] The experiments were run simultaneously in 7 x 10 mL flasks (control and tests) each containing 2 g FW for 10 mL of suspension medium under continuous orbital shaking (60 rpm). Simultaneous changes in pH were measured by using ELIT 808 ionometer with pH sensitive combined electrodes functioning in parallel. Data are expressed as upH and are means ± SE.

Chemicals. Chemicals (Br-cADPR, dantrolene, U73122) were purchased from Sigma (St. Louis, MO). Dantrolene was dissolved in methanol, U73122 was dissolved in DMSO. Final concentration of methanol or DMSO never exceeded 1%.

Statistics. Significant differences between treatments were determined by the Mann and Whitney test and p values <0.05 were considered significant.

Conclusion

A recent in silico re-evaluation of the experimental studies on ABA and $[Ca^{2+}]_{cyt}$ supports that ABA and $[Ca^{2+}]_{cyt}$ increase are decoupled.[13] Even if Ca^{2+} changes can be observed in guard cell in response to ABA, numerous experimental results also show that $[Ca^{2+}]_{cyt}$ increase is only observed in a part of the guard cells population. In response to ABA, absence of $[Ca^{2+}]_{cyt}$ modification does not prevent the occurrence of downstream events such as ion channel regulation[7] and stomatal closure[33] as observed for a weak percentage of cells in our study. Whether or not calcium is an essential signal in stomatal closure has been debated within the field for a number of years.[13] Differential behaviors of anion channels in guard cells among species such as *C. communis, V. faba* and *A. thaliana,* were already reported[34] and gene transcription induced by ABA also differs among different cell types.[35] Given this variability, along with the known Ca^{2+}-independence of some ABA events, questions remain whether ABA-signaling pathways, targets, or models could be generalized for all plant species and all plant cell types, and whether the ABA-induced Ca^{2+}-rise is truly a causative event in ion transport regulation in general and in stomatal closure in particular. However, according to our data, a Ca^{2+} release from intracellular stores could regulate anion channel activity, an early event in an ABA-induced signaling pathway leading to stomatal closure in *A. thaliana*.

Acknowledgements

This work was supported by funds from the MESR to EA 3514. We thank E. Bahin for help in some experiments and T. Kawano for Arabidopsis aequorin seeds.

References

1. Leung J, Giraudat J. Abscisic Acid Signal Transduction. Annu Re Plant Physiol Plant Mol Biol 1998; 49:199-222.
2. Israelsson M, Siegel RS, Young J, Hashimoto M, Iba K, Schroeder JI. Guard cell ABA and CO_2 signaling network updates and Ca^{2+} sensor priming hypothesis. Curr Opin Plant Biol 2006; 9:654-63.
3. Schroeder JI, Allen GJ, Hugouvieux V, Kwak JM, Waner D. Guard cell signal transduction. Annu Rev Plant Physiol Plant Mol Biol 2001; 52:627-58.
4. Roelfsema MR, Hedrich R. In the light of stomatal opening: new insights into 'the Watergate'. New Phytol 2005; 167:665-91.
5. Blatt MR. Potassium channel currents in intact stomatal guard cells: rapid enhancement by abscisic acid. Planta 1990; 180:445-55.
6. Allen GJ, Kuchitsu K, Chu SP, Murata Y, Schroeder JI. Arabidopsis abi1-1 and abi2-1 phosphatase mutations reduce abscisic acid-induced cytoplasmic calcium rises in guard cells. Plant Cell 1999; 11:1785-98.
7. Levchenko V, Konrad KR, Dietrich P, Roelfsema MR, Hedrich R. Cytosolic abscisic acid activates guard cell anion channels without preceding Ca^{2+} signals. Proc Natl Acad Sci USA 2005; 102:4203-8.
8. Hetherington AM. Guard cell signalling. Cell 2001; 107:711-4.
9. Garcia-Mata C, Gay R, Sokolovski S, Hills A, Lamattina L, Blatt MR. Nitric oxide regulates K^+ and Cl^- channels in guard cells through a subset of abscisic acid-evoked signaling pathways. Proc Natl Acad Sci USA 2003; 100:11116-21.
10. Jones AM, Assmann SM. Plants: the latest model system for G-protein research. EMBO Report 2004; 5:572-8.
11. McAinsh MR, Browlee A, Hetherington AM. Abscisic acid-induced elevation of guard cell cytosolic Ca^{2+} precedes stomatal closure. Nature 1990; 343:186-8.
12. Gilroy S, Read ND, Trewavas AJ. Elevation of cytoplasmic calcium by caged calcium or caged inositol triphosphate initiates stomatal closure. Nature 1990; 346:769-71.
13. Li S, Assmann SM, Albert R. Predicting essential components of signal transduction networks: a dynamic model of guard cell abscisic acid signaling. PLoS Biol 2006; 4:312.
14. Kinoshita T, Nishimura M, Shimazaki K. Cytosolic concentration of Ca^{2+} regulates the plasma membrane H^+-ATPase in guard cells of fava bean. Plant Cell 1995; 7:1333-42.
15. MacRobbie EA. ABA activates multiple Ca^{2+} fluxes in stomatal guard cells, triggering vacuolar K^+, Rb^+ release. Proc Natl Acad Sci USA 2000; 97:12361-8.
16. Hetherington AM, Brownlee C. The generation of Ca^{2+} signals in plants. Annu Rev Plant Biol 2004; 55:401-27.
17. Wu Y, Kuzma J, Maréchal E, Graeff R, Lee HC, Foster R, et al. Abscisic acid signaling through cyclic ADP-ribose in plants. Science 1997; 278:2126-30.
18. Marius P, Guerra MT, Nathanson MH, Ehrlich BE, Leite MF. Calcium release from ryanodine receptors in the nucleoplasmic reticulum. Cell Calcium 2006; 39:65-73.
19. Staxen I, Pical C, Montgomery LT, Gray JE, Hetherington AM, McAinsh MR. Abscisic acid induces oscillations in guard-cell cytosolic free calcium that involve phosphoinositide-specific phospholipase C. Proc Natl Acad Sci USA 1999; 96:1779-84.
20. Sánchez JP, Duque P, Chua NH. ABA activates ADPR cyclase and cADPR induces a subset of ABA-responsive genes in Arabidopsis. Plant J 2004; 38:381-95.
21. Leckie CP, McAinsh MR, Allen GJ, Sanders D, Hetherington AM. Abscisic acid-induced stomatal closure mediated by cyclic ADP-ribose. Proc Natl Acad Sci USA 1998; 95:15837-42.
22. Brault M, Amiar Z, Pennarun AM, Monestiez M, Zhang Z, Cornel D, et al. Plasma membrane depolarization induced by abscisic acid in Arabidopsis suspension cells involves reduction of proton pumping in addition to anion channel activation, which are both Ca^{2+} dependent. Plant Physiol 2004; 135:231-43.
23. Trouverie J, Vidal G, Zhang Z, Sirichandra C, Madiona K, Amiar Z, et al. Anion channel activation and proton pumping inhibition involved in the plasma membrane depolarization induced by ABA in *Arabidopsis thaliana* suspension cells are both ROS dependent. Plant Cell Physiol 2008; 49:1495-507.
24. Wang P, Song CP. Guard-cell signalling for hydrogen peroxide and abscisic acid. New Phytol 2008; 178:703-18.
25. Kobayashi S, Bannister ML, Gangopadhyay JP, Hamada T, Parness J, Ikemoto N. Dantrolene stabilizes domain interactions within the ryanodine receptor. J Biol Chem 2005; 280:6580-7.
26. Mikoshiba K. The IP_3 receptor/Ca^{2+} channel and its cellular function. Biochem Soc Symp 2007; 74:9-22.
27. Xu L, Tripathy A, Pasek DA, Meissner G. Potential for pharmacology of ryanodine receptor/calcium release channels. Ann NY Acad Sci 1998; 16:130-48.
28. Kadono T, Yamaguchi Y, Furuichi T, Hirono M, Garrec JP, Kawano T. Ozone-Induced Cell Death Mediated with Oxidative and Calcium Signaling Pathways in Tobacco Bel-W3 and Bel-B Cell Suspension Cultures. Plant Signal Behav 2006; 1:312-22.
29. Sutton F, Paul SS, Wang XQ, Assmann SM. Distinct abscisic acid signaling pathways for modulation of guard cell versus mesophyll cell potassium channels revealed by expression studies in *Xenopus laevis* oocytes. Plant Physiol 2000; 124:223-30.
30. Reboutier D, Bianchi M, Brault M, Roux C, Dauphin A, Rona JP, et al. The indolic compound hypaphorine produced by ectomycorrhizal fungus interferes with auxin action and evokes early responses in nonhost *Arabidopsis thaliana*. Mol Plant Microbe Interac 2002; 15:932-8.
31. Goh CH, Kinoshita T, Oku T, Shimazaki K. Inhibition of blue light-dependent H^+ pumping by abscisic acid in Vicia guard-cell protoplasts. Plant Physiol 1996; 11:433-40.
32. Pugin A, Frachisse JM, Tavernier E, Bligny R, Gout E, Douce R, et al. Early Events Induced by the Elicitor Cryptogein in Tobacco Cells: Involvement of a Plasma Membrane NADPH Oxidase and Activation of Glycolysis and the Pentose Phosphate pathway. Plant Cell 1997; 9:2077-91.
33. Gilroy S, Fricker MD, Read ND, Trewavas AJ. Role of calcium in signal transduction of Commelina guard cells. Plant Cell 1991; 3:333-44.
34. Forestier C, Bouteau F, Leonhardt N, Vavasseur A. Pharmacological properties of slow anion currents in intact guard cells of Arabidopsis. Application of the discontinuous single-electrode voltage-clamp to different species. Pflug Arch 1998; 436:920-7.
35. Leonhardt N, Kwak JM, Robert N, Waner D, Leonhardt G, Schroeder JI. Microarray expression analyses of Arabidopsis guard cells and isolation of a recessive abscisic acid hypersensitive protein phosphatase 2C mutant. Plant Cell 2004; 16:596-615.